A STUDY OF THE POSTERIOR NUCLEAR FORMS OF *TRYPANOSOMA RHODESIENSE* (STEPHENS AND FANTHAM) IN RATS

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Trypanosomes, obtained from a human source, presenting a posterior position of the macronucleus were first described by Stephens and Fantham.[1] Upon this morphological peculiarity they founded their species *Trypanosoma rhodesiense*. Since the publication of their paper, a similar condition has been described in trypanosomes from human sources by various observers in different regions. The patient whose strain they described contracted the disease in a portion of Rhodesia in which the absence of *Glossina palpalis* rendered it necessary to seek a different carrier. Stannus and Yorke[2] discovered posterior nuclear forms in a strain taken from a case of human trypanosomiasis in Nyasaland. More recently Stannus[3] has noted the presence of these forms in a strain obtained from a case of human trypanosomiasis from Portuguese East Africa, while the Royal Society's Commission[4] confirms the observation of Stannus and Yorke with regard to the presence of posterior nuclear forms in strains from human trypanosomiasis in Nyasaland. The Commission bears out their conclusion that the trypanosome of the human trypanosome disease of Nyasaland is probably identical with *T. rhodesiense*.

The occurrence of posterior nuclear forms is not, however, confined to strains of human trypanosomiasis. Such forms have also been recorded among animal trypanosomiases, for example in *T. pecaudi* by Wenyon,[5] in a strain of *T. equiperdum* by Yorke and Blacklock,[6] and in a strain of *T. brucei* from Uganda by Blacklock.[7]

THE INCIDENCE OF POSTERIOR NUCLEAR FORMS IN *T. RHODESIENSE*

Stephens and Fantham (loc. cit.) observed that the forms of trypanosome which presented a posterior position of the nucleus were the short and stumpy ones, and that in rats forms having this peculiarity made their appearance in the peripheral blood about the fifth or sixth day of the disease, and increased in number to the seventh or eleventh day, when they formed about 6 % of the parasites present.

SHORT AND STUMPY

The term 'short and stumpy' appears to be used in somewhat different senses by different observers, in relation to trypanosomes. For example, Bruce (loc. cit.) says 'There is no free flagellum in the short and stumpy forms.' In a table attached, however, he shows that the amount of free flagellum in the 'short and stumpy' forms (620 individuals) averages 0·6. Stephens and Fantham (loc. cit.) say of the stumpy forms of *T. rhodesiense* which have a posterior nucleus that there is a well-marked blepharoplast and *a very short free flagellum.* It appears, therefore, to be not quite decided what constitutes a 'short and stumpy' form. Is it a trypanosome which measures less than a given number of microns, e.g., 21; is it a trypanosome which has absolutely no free flagellum; or is it a trypanosome which is not only less than a certain length, but also has absolutely no free flagellum? In the experiments given below the large majority of forms which had a posterior nucleus measured less than 21 μ, but numbers were found which exceeded this length. Again, of the parasites which conformed to the definition as regards shortness, numbers had absolutely no free flagellum, while numbers had a very definite portion of flagellum free. In some cases this portion was of considerable length. It was not impossible, for example, to discover forms which while they measured only 19 μ in length, yet possessed a free portion of flagellum amounting to 4 μ.

One finds thus that when studying the posterior nuclear forms in these experiments, one is not dealing with short forms in the strict sense that they measure in all cases less than 21 μ nor with

stumpy forms in the sense that they possess absolutely no free flagellum. There are overlapping forms which do no come under both categories, in fact there are a few which come under neither, yet present the posterior nucleus. For practical purposes, however, it may be said that the posterior nuclear forms, whether free-flagellated or aflagellar, which exceed the measurement of 'short forms,' were few in number. The experiments detailed below were undertaken in order to determine in a given infection in rats the time of appearance of posterior nuclear forms in the peripheral blood, and the numerical relationship which these forms bear to other forms of trypanosome present from day to day.

METHOD OF CARRYING OUT THE EXPERIMENTS

A guinea-pig infected with *T. rhodesiense* and having numerous parasites in its blood was utilized as the source of infective material.

Four groups (A, B, C, D) of rats, each group containing six rats, were selected, the rats being as nearly as possible of the same weight. The parasites per cubic millimetre in the blood of the guinea-pig were estimated by means of the Thoma Zeiss haemocytometer, and dilutions made in a warmed mixture of 1 % sodium citrate and 0·85 % sodium chloride. The dilutions were made so that the rats of the first two groups received 1,000,000 trypanosomes, the rats of the third group 4,000,000, and the rats of the fourth group 8,000,000. The animals were all inoculated intraperitoneally, the amount of the injection in each case being 0·5 c.c.

INCUBATION AND DURATION

The average incubation period of Group A (1,000,000 trypanosomes) was 4·8 days, the average duration of the disease 13·6 days. Group D (8,000,000 trypanosomes) had an average incubation period of 4·5 days and an average duration of 15·5 days. Table I gives details as to the incubation and duration of the disease in the individuals of each group. From this table it appears that no definite variation in either incubation or duration could be attributed to the relative numbers of trypanosomes injected.

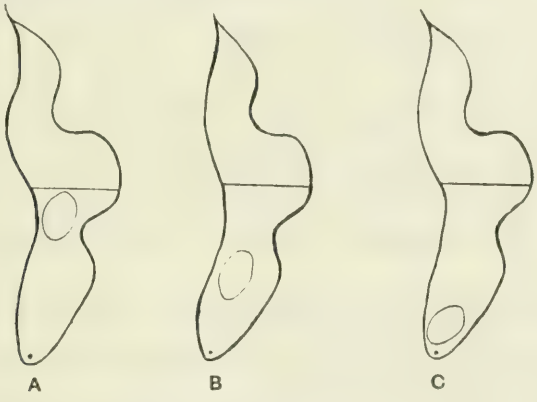
TABLE I.—Giving incubation and duration of infection with *T. rhodesiense* in 24 rats.

Group	No. of Experiment	Incubation in days	Duration
A	Rat 2231 A	5	13
1,000,000	,, B	4	13
Trypanosomes inoculated	,, C	5	14
	,, D	6	14
	,, E	4	14
	,, F	5	14
B	Rat 2232 A	5	13
1,000,000	,, B	6	13
	,, C	5	16
	,, D	5	16
	,, E	4	13
	,, F	6	13
C	Rat 2229 A	5	13
4,000,000	,, B	4	12
	,, C	4	11
	,, D	4	10
	,, E	4	13
	,, F	6	13
D	Rat 2230 A	4	17
8,000,000	,, B	4	17
	,, C	4	15
	,, D	5	14
	,, E	5	15
	,, F	5	15
	Average ...	4·7	13·7

ENUMERATIONS

From the day on which parasites first appeared in the peripheral blood until the death of the animal, a film was examined daily in the fresh state, and a thin film dried and stained. The dried films were fixed in absolute alcohol and stained with Giemsa's stain.

1. *Preliminary count.* In each stained film a count of 200 parasites was made wherever possible. Every trypanosome met with was counted, whether long, intermediate or short, dividing or non-dividing. The number of posterior nuclears was noted, non-dividing forms only being chosen. The result, therefore, gave the number of non-dividing posterior nuclear forms per 200 of all forms. The posterior nuclear forms were classified carefully according to the position of the nucleus. Those forms were classified as A in which the nucleus, although definitely posterior to the centre of the parasite (excluding free flagellum) still lay close to the centre. C forms were those in which the nucleus lay adjacent to the blepharoplast, while B forms were intermediate in position. The drawings (Text-fig. 1) give the positions indicated for each group.



Text-Fig. 1. Showing various positions of nucleus. A. B. C.

RESULT OF THE PRELIMINARY COUNT

The first trypanosomes which appeared in the peripheral blood of each rat were of the long and slender, or intermediate types. Subsequently, short forms made their appearance, and after a few days posterior nuclear forms. In no case was a posterior nucleated

form found in the first day's count of 200 in any rat. The posterior nuclear forms made their appearance in the peripheral blood with considerable regularity, in the following order:— first A forms, next B forms, and lastly C forms. Thus of the twenty-four rats, A forms appeared first in thirteen, B forms first in six. In four, A and B forms appeared together, while in one, A and C forms appeared together. In none of the rats was a C form the first type of posterior nuclear to appear.

DAY ON WHICH POSTERIOR NUCLEAR FORMS FIRST APPEARED

The earliest day of the disease on which posterior nuclear forms were first seen in the peripheral blood in this count was the seventh, the latest on which they first appeared was the thirteenth. The average day of their appearance in the twenty-four rats was the ninth day of the disease. From the time of their first appearing, posterior nuclear forms were usually to be found up to the death of the animal. It was observed that the posterior nuclears increased not only actually as the disease progressed, but also relatively to other forms of parasite. The greatest number of posterior nuclear forms in any 200 counted occurred on the day of death in fourteen out of the twenty-four rats, on the day preceding the day of death in seven rats, two days before the day of death in two rats and previous to this day in the remaining rat.

From the enumeration of the parasites in films from the infected animals by this preliminary count, the results obtained were as follows:—

(1) The first forms of parasite found present in the peripheral blood were long and intermediate free flagellated forms.

(2) Short forms appeared later.

(3) Posterior nucleated forms only appeared after the disease had developed somewhat.

(4) Posterior nucleated forms increased in numbers from the time of their appearance, both actually and relatively to other forms of trypanosome.

(5) Of the posterior nuclear forms those with the nucleus near the centre of the trypanosome appeared first, those with the nucleus

near the blepharoplast last, while the forms with the nucleus between those two extremes made their appearance at an intermediate stage of the disease.

THE CONSTANCY OF APPEARANCE OF POSTERIOR NUCLEAR FORMS IN THESE EXPERIMENTS

Laveran[8] states that the morphological peculiarity of *T. rhodesiense* (posterior nuclears) in rats and mice is not constant. In these experiments, comprising twenty-four rats, there was no exception. Posterior nuclear forms were found in all cases in the preliminary count. Two rats only did not present the advanced form (C form) during this count, but this form was easily found on further search.

2. *Second count.* Two rats out of each group, each having a good infection, were chosen for the purposes of this count. A thousand trypanosomes were enumerated whenever possible in each daily film, and the posterior nuclear forms noted as before. The results of this more laborious procedure confirmed the conclusions arrived at as the result of the first examination, the earliest day on which posterior nuclears were first found being the sixth to the tenth. It appeared also that C forms occurred in greater proportion in those animals which lived the longest time, and that within the group of posterior nuclear forms the C forms generally increased relatively to the A and B forms towards the end of the disease. Table II gives an illustration of some of these points; it gives the number of posterior nuclear forms of the three kinds per thousand of trypanosomes during the course of the disease in two rats.

POST-MORTEM RELATIVE INCREASE OF POSTERIOR NUCLEAR FORMS

In several of the rats from which films were taken after death it was observed that the proportion of posterior nuclear to other forms increased considerably. This phenomenon appears to be related to the observation that frequently after death the 'short stumpy' forms of parasite in *T. rhodesiense* resist the processes of disintegration in the blood of the dead host better than the long forms. Bevan and MacGregor[9] drew attention to this fact. It has also been recorded by Swellengrebel[10] in the mouse, and by Blacklock[11] in the rat.

TABLE II.—To show the dates of appearance of posterior nuclear forms of various grades in the peripheral bood of two rats infected with *T. rhodesiense*, — per 1000 trypanosomes.

Date	Rat 2230 C			Rat 2231 A		
	A	B	C	A	B	C
Oct. 4 ... (Inoculation)	—	—	—	—	—	—
,, 5 ...	—	—	—	—	—	—
,, 6 ...	—	—	—	—	—	—
,, 7 ...	—	—	—	—	—	—
,, 8 ...	—	—	—	—	—	—
,, 9 ...	1	—	—	*—	—	—
,, 10 ...	6	3	—	1	—	—
,, 11 ...	2	2	—	1	—	—
,, 12 ...	*—	—	—	4	2	—
,, 13 ...	1	—	1	—	—	—
,, 14 ...	1	—	2	4	—	—
,, 15 ...	—	1	1	6	2	1
,, 16 ...	4	3	3	10	5	4
,, 17 ...	2	—	1	8	1	1
,, 18 ...	14	12	6	7	1	5
,, 19 ...	6	27	21	—	—	—
,, 19† ...	32	31	22	—	—	—

* Less than 1000 counted on film.

† After death of rat.

THE SIGNIFICANCE OF POSTERIOR NUCLEAR FORMS IN *T. RHODESIENSE*

Many explanations have already been advanced to account for these forms of parasite. Bevan[12] states that the presence of short forms of trypanosome with the macronucleus slightly posterior to the centre is a common feature in many species, and especially in trypanosomes undergoing degeneration or taking on the resistant form in the presence of adverse or unusual conditions. This

explanation does not suffice to explain the typical posterior nuclear forms in *T. rhodesiense*, because in this species it is not a case of the nucleus being *slightly posterior to the centre* merely. There are certainly many forms which conform to this description, but the typical posterior nuclear forms do not fall under this category; they are something much more definite. As regards the suggestion that they are the product of degeneration, it is difficult to explain why they are not found when *T. gambiense* degenerates. As previously stated, there is some evidence that such forms are capable of great resistance to the processes of disintegration in the cadaver. It appears improbable that the same phenomenon, 'posterior nuclears,' could be evidence at one and the same time of degeneration and resistance: it is possible, of course, that the trypanosomes may assume this arrangement of the nucleus in response to the demands of an unsuitable environment, and that subsequently if the environment continues to be unsuitable they degenerate, still retaining this arrangement of the nucleus. But the study of *T. gambiense* under conditions where the parasites are obviously degenerating has not, up to the present, led to the discovery of such forms.

ABERRANT FORMS

Bruce (loc. cit.) refers to the posterior nuclear forms seen by him in the *T. rhodesiense* of Nyasaland as 'aberrant' forms. In view, however, of the fact that they are so constant in their appearance in this strain and form an integral part of it, it seems hardly justifiable to treat them merely as 'aberrant' forms.

DUE TO TECHNIQUE

It has been stated that they may be due to methods of taking films, fixing or staining, but so many observers have noted their appearance, using different methods of treatment for the films, that it seems reasonable to discard this view.

In seeking for an explanation of their presence, it is essential to discover, if possible, whether or not they ever occur in *T. gambiense*. Up to the present, so far as one is aware, they have not been described in this trypanosome. It may be argued

that the number of *T. gambiense* strains which has been examined is small. If, however, it can be established that they are entirely absent from *T. gambiense*, it will be difficult to attribute their presence in *T. rhodesiense* to such changes as degeneration, or to resistance, or to relegate them to the class of 'aberrant' forms. If further examination of strains of *T. gambiense* fails to reveal their presence, it must be concluded that these forms constitute an important distinction between the two parasites *T. gambiense* and *T. rhodesiense*. The fact that such forms are found in animal strains does not diminish their claim to attention, since animals are known to be infected with human trypanosomes. The argument, that if so many animal strains were capable of infecting human beings there would be a great amount of human trypanosomiasis in regions where it does not at present abound, is not conclusive. It has to be definitely proved that there is actually no human trypanosomiasis in such animal-infected regions. The experience in Nyasaland, and in Rhodesia, Northern and Southern, renders it necessary to be cautious in considering a region free from sleeping sickness. Even if human cases were rare, it may fairly be argued that we do not by any means fully understand the factors that govern the successful infection of human beings.

FLY VARIATION

If we regard *T. rhodesiense* simply as a variation of the human parasite of the Gambia, it is possible that the explanation of their occurrence is to be sought, not so much in the conditions of their environment in the blood of human beings and in that of inoculated animals, as in other factors, for example, the transmitting agent, the fly. Against this hypothesis we have the facts that although *T. vivax* and *T. pecorum* are each known to be transmitted by both *G. palpalis*[13] and *G. morsitans*,[14] they do not appear to be modified morphologically by the difference of carrier.

It would be of considerable interest to ascertain whether in cases where *T. gambiense* is transmitted by *G. morsitans* such posterior nuclear forms are to be found in the blood of infected animals.

MULTIPLE INFECTION

The possibility of there being an infection in human beings with more than one species of trypanosome suggests itself. In favour of this one might adduce the facts that not only do animals in nature frequently suffer from mixed infections, but also that certain species of fly have proved themselves capable of transmitting more than one species of trypanosome. It seems probable that human beings are susceptible to mixed infection. The proof and demonstration of a double infection, given that the human being harboured species which were each universally pathogenic to laboratory animals would, with the means at present at our disposal, be practically impossible. In this connection one might refer to the original Gambian Horse Trypanosome described by Dutton and Todd[15]; in this case infection was considered to be caused by a single species of trypanosome, a view which is not now generally accepted. It is only where the pathogenicity of the trypanosomes taking part in a mixed infection is widely divergent as regards laboratory animals that proof of the presence of more than one sepcies of parasite can be given.

Against the hypothesis of mixed infection in the case of *T. rhodesiense* is the evidence derived from transmission experiments. Kinghorn and Yorke,[16] transmitting *T. rhodesiense* by means of *Glossina morsitans* bred in the laboratory, found posterior nuclear forms in all animals which became infected. This implies, accepting for a moment the mixed infection hypothesis, that the flies which became infected acquired and passed on the mixed infection in each case. This is, perhaps, the strongest argument against the hypothesis. A further argument against the possibility is somewhat of the same order, namely, the results of infection by culture of *T. rhodesiense*, which Bayon[17] records.

CONCLUSIONS

1. Posterior nuclear forms first appear in the blood of rats infected with *T. rhodesiense* from the sixth to the tenth day of the disease, taking a count of a thousand trypanosomes.
2. They increase in numbers in the later stage of the disease.
3. They increase relatively to other forms of trypanosome.

4. They cannot be explained as the result of either faulty technique or degeneration.

5. They show definite powers of resistance to disintegration in the cadaver of the animal host.

6. The presence of posterior nuclear forms may be due to:—

(*a*) The occurrence of such forms as a constant constituent of certain strains.

(*b*) A mixed infection.

(*c*) Certain unexplained influences in the blood environment, affecting the parasites.

(*d*) The transmitting agent.

7. There are, at present, stronger arguments against the last three explanations of their presence than against the first.

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